Neuromuscular junctions: **The state of the union**

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Synaptic differentiation is triggered by signals from the ingrowing axon and is shaped by information exchange between the presynaptic and postsynaptic cells. The central role of agrin in this process, and the identity of the signaling component of its receptor, have now been established.

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Efficient communication is the foundation of any productive union. At synapses, signaling relies on the regulated and localized secretion of neurotransmitters by the nerve terminal, and the selective accumulation of their cognate receptors on the surface of the receiving cell [1]. For example, acetylcholine receptors at neuromuscular junctions are several thousand-fold more concentrated in the postsynaptic apparatus than in non-synaptic membranes of the same cell. The ingredients of a successful synapse are clear, but what about the molecular mechanisms that induce their differentiation? Three recent papers [2–4] reveal a great deal about these mechanisms, and show that they are fundamentally unified through the actions of agrin and the signaling component of its receptor, MuSK (muscle specific kinase).

Early experiments by McMahan and colleagues (reviewed in [5]) showed that the basal lamina at neuromuscular junctions contains information that can direct both presynaptic and postsynaptic differentiation. Agrin is a component of this specialized extracellular matrix, and agrin's benchmark biological activity is its ability to induce acetylcholine receptor clusters on cultured myotubes (reviewed in [5,6]). This biological activity is largely restricted to a subset of agrin isoforms that are expressed only in neurons [7]. These and other observations led to the hypothesis that agrin, released by motor neurons, induces the differentiation of the postsynaptic membrane on the muscle cell [8]. But two key questions remained open: what is agrin's role in the intact animal, and how does it signal?

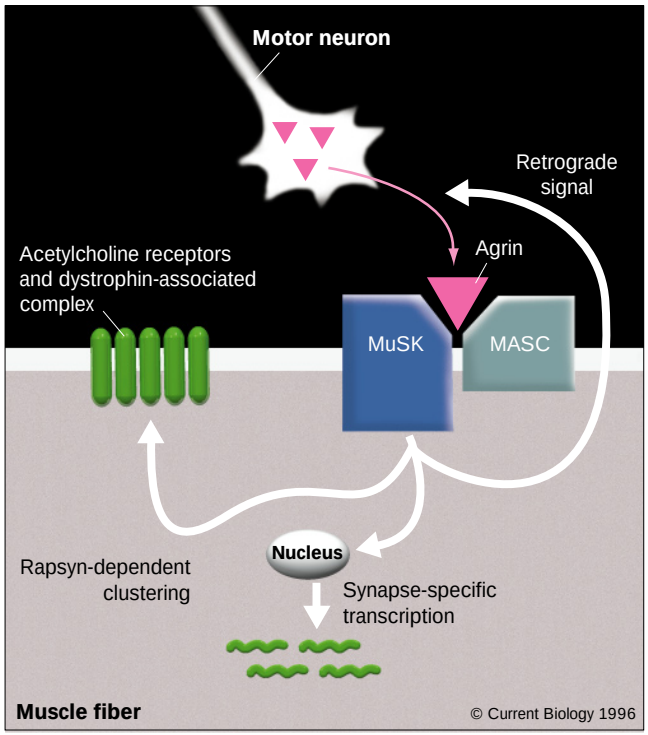
Joshua Sanes' group used targeted disruption of the agrin gene to address the first question [2]. As predicted, mice lacking agrin (all isoforms are affected in the mutant) showed sharply reduced numbers of motor endplates. Interestingly, the mice did exhibit limited postsynaptic differentiation, suggesting that the machinery for clustering acetylcholine receptors is conserved even in the absence of agrin. Indeed, myotubes cultured from these mutant mice responded vigorously to wild-type agrin [2]. Quite unexpectedly, the phenotype reached beyond the postsynaptic cell to include major presynaptic deficits as well. These satisfying results place agrin squarely at the center of synaptic differentiation at the neuromuscular junction.

How does agrin work? Because it is a secreted matrix molecule, agrin must have a cell-surface receptor through which it exerts its effects. Two important clues to the nature of this receptor came from the observation that agrin induces tyrosine phosphorylation of acetylcholine receptors, and that a host of other postsynaptic molecules also redistribute in response to agrin [9]. Both clues were built upon in an elegant set of experiments from George Yancopoulos' group [3,4]. They had previously identified an 'orphan' receptor tyrosine kinase, MuSK, that is selectively expressed at the neuromuscular junction. In the recent work, they found that MuSK is activated rapidly and exclusively by the highly active (neural) agrin isoforms [3]. Targeted disruption of the MuSK gene yielded a phenotype remarkably similar to that of the agrin knockouts, but the effects were even more severe: synapses failed to form, acetylcholine receptor clusters were nonexistent, and myotubes cultured from these mice did not respond to agrin [4].

Although MuSK is essential for mediating agrin signals, it does not act alone. Isolated MuSK does not bind agrin. Furthermore, MuSK expressed on the cell surface can only be activated by (and cross-linked to) agrin in the context of a differentiated muscle cell. The Yancopoulos group thus hypothesize that there is a second element to the agrin receptor, dubbed MASC (myotube-associated specificity component) [3]. One candidate for MASC is α-dystroglycan, a membrane-associated molecule that binds agrin and is expressed on muscle cells. However, the major muscle forms of α-dystroglycan do not show the predicted selectivity for agrin isoforms, at least when tested in isolation. Moreover, fragments of agrin that do not bind these α-dystroglycans nevertheless retain the ability to induce acetylcholine receptor phosphorylation and clustering, albeit with much reduced efficacy [10]. But, because α-dystroglycans from brain can demonstrate ligand selectivity [11], the potential for agrin isoform-selective, MASC-like dystroglycans in muscle should not be ruled out. Nonetheless, MASC's identity remains very much an open question.

Together, these new findings suggest that agrin activation of MuSK initiates as many as three distinct signaling

Figure 1



Differentiation of the neuromuscular junction is initiated by the release of agrin from the ingrowing axons, and proceeds by at least three pathways. All of the pathways are initiated through the activation of a receptor complex that includes MuSK and an unidentified accessory component, MASC. One path leads to the clustering of acetylcholine receptors, MuSK itself, and the dystrophin-associated protein complex. Separately, MuSK activation by agrin is also required for the synapse-specific transcription of acetylcholine receptors and for the expression of retrograde signals mediating presynaptic differentiation.

pathways in synaptic differentiation (Fig. 1). The best understood of these mediates clustering of acetylcholine receptors, dystrophin/utrophin-associated proteins (including α-dystroglycan) and other elements of the postsynaptic apparatus. This path is distinguished by its requirement for the cytosolic, acetylcholine receptor-associated protein rapsyn [12]. Interestingly, rapsyn overexpression in cultured fibroblasts leads to MuSK activation and the clustering of acetylcholine receptors in an agrin-independent fashion [13], suggesting a pathway for the 'inside-out' regulation of postsynaptic architecture.

A second pathway, revealed for the first time by the recent work, leads to the organization of the machinery needed for synapse-selective transcription. In mature muscle, subsynaptic nuclei preferentially transcribe mRNA encoding the acetylcholine receptor, and this expression is regulated by neurally derived factors. ARIA (acetylcholine receptor-inducing activity), a member of the neuregulin family of signaling molecules, is likely to be one such factor [14]. ARIA is released from the motorneuron and acts *via* erbB receptor tyrosine kinases, which themselves are aggregated at the endplate. Remarkably, both erbB clustering and synapse-selective transcription are disrupted in MuSK and agrin knockout animals.

A third arm of the agrin–MuSK signaling pathway, which could act either in sequence with or in parallel to the second, is suggested by the aberrant presynaptic differentiation in the mutant mice. Bundled motor axons normally course through the muscle, with individual axons peeling off and terminating at endplates in the central one-third of the myofibers. In MuSK mutant mice, axons fail to terminate and extend over the entire surface of the muscle, showing no evidence of presynaptic specializations [4]. The agrin mutant mice show a similar, though less dramatic, phenotype [2], whereas the rapsyn knockout mice show a milder phenotype in this regard. The agrin–MuSK signal is therefore an essential step in the elaboration of a retrograde signal instructing the axon to stop and differentiate into a presynaptic terminal. Interestingly, recent work suggests that agrin itself could be one such signal: isoforms of agrin synthesized by muscle, which are selectively secreted at developing postsynaptic specializations, can act as a 'stop signal' for growing axons in culture [15,16].

The biological activities of agrin, and their underlying signal transduction pathways, are far richer and more complex than expected. What is next? The identity of MASC, and the steps between MuSK activation and its myriad effector arms are obvious targets for attention. In addition, the agrin isoforms that activate MuSK in muscle cells are also expressed in many non-motor neurons of the central nervous system [17]. As MuSK and related receptors are also expressed in the central nervous system, these pathways may find their way to the brain.

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